PROCEDURES FOR SUBRENAL ASSAYS USING HUMAN TUMOR XENOGRAFTS

ANIMALS:

Propagation and Testing:	Athymic Swiss(Cr:NIH(S)-nu)or athymic random bred (NCr-nu) mice.
Weight:	Mice should have a minimum weight of 18 gm for males and 17 gm for females.
Age:	Record age of mice.
Sex:	One sex is used for all test and control animals in one experiment.
Source:	one source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

EXPERIMENT SIZE:

General Testing:	Six animals per test group and 12 animals per control group. When an early control group is required, 10 additional animals should be added to the control.

TUMOR TESTING:

Fragment:

Size 10^3	Prepare 10x10x10 Ocular Micrometer Unit (OMU) fragments. Average diameter must be 9-l 2 OMU's measured under a dissecting microscope.
Size 3.93	Prepare 19x19x19 Ocular Micrometer Unit (OMU) fragments. Average diameter must be 17-21 measured under a dissecting microscope. 10 OMU's = 1 mm.

Anesthetic: Any satisfactory anesthetic (e.g., chloral hydrate, Avertin, etc.).

Medium: Tissue culture medium with no antibiotics (e.g., 199, Eagles MEM, or Earles).

Site:	Implant fragment under the subrenal capsule using a 16-gauge trocar with a 226 bevel, after exposing the kidney with a 7 mm dorsal skin incision. The wound is closed with a 9 mm wound clip after closing the peritoneum with l-4 silk sutures.
Early Control:	When early control groups are required, on the day designated (usually Day 4) record body weights (initial weigh Day). Sacrifice early control group, measure tumors in OMU's. Calculate mean tumor weight. Record this mean tumor weight as the initial tumor weight for control group. To provide estimated initial measurements for the treated groups, eliminate the measurements for the two largest and the two smallest tumors in the early control group. The mean tumor weight of the remaining six mice is designated the initial weight for all treated groups. Initiate test agent injections on this day for studies that require early controls.
Weigh Days:	The Initial Weigh Dav is day of implant except for those studies that require an early control. The initial weigh day is the day of sacrifice of the early control group in those studies. The Final Weigh Day is the evaluation day for all studies.

QUALITY CONTROL:

(1) Implant 2 or 3 additional mice which can be used for replacements in the event of surgical deaths. If surgical deaths do not occur, use these mice as additional control animals.

(2) Within a given experiment, whenever possible use mice from the same supplier, date of receipt, and shipping crate to reduce fighting. If mice fight, house fighters individually.

(3) Donor tumor should weigh between 500-800 mg and be scrupulously cleaned of necrotic and/or hemorrhagic areas.

(4) Run bacterial cultures.

(5) Record deaths daily. In case of unusual deaths, these animals should be autopsied and peculiarities noted.

(6) Specific definitions for subrenal capsule implants.

(a) Acceptable control mean tumor weight change is equal to or greater than one mass doubling or a 0.3 log,, increase between Day 0 and Final Evaluation Day.

(b) Control no-take: A mouse with a tumor weight increase of <20% between Day

0 and Final Evaluation Day.

(c) Excessive control no-takes: 4 or more no takes are excessive in a control group of 12 mice.

(d) Excessive control early deaths: 2 or more control deaths in a group of 10 to 12 animals (i.e.,>lO%) on or before Final Evaluation Day that are not attributable to surgery or accident.

EVALUATION: (refer to Protocol 11)

The parameter measured is mean tumor weight change (delta) based on length and width measurements in millimeters. Compute mean animal body weights for Weigh Days 1 and 2, Compute T/C for all test groups with >67% survivors on Final Evaluation Day. An excessive body weight loss >=5.0 g may be used in evaluating toxicity.

On Day 0 and on Final Evaluation Day, measure and record length and width measurements for tumors. The dimensions are measured and recorded in OMU'S. By convention, the length (L) dimension must be entered first.

(1) Convert OMU's to millimeters (mm).

(2) Calculate tumor weights (mgs) from tumor dimensions (mm x mm) following the formula for the volume of a prolate ellipsoid:

$\frac{L \times W^2}{2}$ where L is the longer of the two 2 measurements.

(3) Calculate the change (delta) in mean tumor weight for each group of mice:

Change in Mean Tumor Weight =

Mean Tumor Weight FINAL - Mean Tumor Weight INITIAL.

(4) Calculate the change (delta) in mean tumor weight for test (T) and control (C) groups.

(5) Calculate T/C% for all test groups with >67% survivors on Final Evaluation Day:

$$T/C\% = \frac{\Delta WtT}{\Delta WtC} \times 100 \text{ -- if } \Delta WtT \text{ positive.}$$

$$T/C\% = \frac{\Delta WtT}{\text{Test Mean Tumor Weight INITIAL}} \times 100 \text{ -- if } \Delta WtT \text{ is negative.}$$

REPORT OF DATA:

Assign a code of to an individual mouse whose response is considered invalid, including the following circumstances:

(1) tumor lost from site of implant and kidney appears normal.

(2) Accidental injury or death.

(3) More than 1 tumor present.

(4) Infection at site of implant.

(5) Kidney does not appear normal.

SPECIFIC TUMOR SUBRENAL CAPSULE PROTOCOL

	Tumor Code	Tumor Line	Doubling Times SC	Doubling Times SRC	Implant Size (OMU)	Initial Meas. Day	Weigh Days	Rx (Days)	Evaluation Day	Schedule Version	Schedule Date
LUNG (NSCL)											
	QS	EKVX	3.6-7.9	3.3-3.4	10(3)	0	0,13	2,6,10	13	A	07/22/91
	CL	NCI-H460	1.9-2.9	1.6-3.2	10(3)	0	0,9	2,6	9	A	04/30/87
	LN	A549	5.8	2.8-7.3	10(3)	0	0,12	1,5,9	12	A	11/25/85
					19(3)	4*	4,19	4,8,12,16	19	B(Rev)	07/24/91
	BB	HOP-92	3.3-8.4	2.7-11	19(3)	0	0,13	2,6,10	1	A	07/22/91
	JA	NCI-H23	4.2-7.5	2.3-5.7	19(3)	4*	4,15	4,8,12	15	B	04/27/87
	LV	NCI-H322M	3.2-7.6	6.1-27	19(3)	4*	4,19	4,8,12,16	19	B	06/16/87
	JC	NCI-H522	2.3-5.8	2.1-3.1	19(3)	4*	4,15	4,8,12	15	A	11/14/86
					19(3)	0	0,14	2,6,10	13	B(Rev)	07/23/91
					10(3)	0	0,13	2,6,10	13	C(Rev)	02/21/92
	JG	NCI-H520	3.8-5.5	2.5-3.9	19(3)	4*	4,11	4,8	11	B	04/30/87
					19(3)	0	0,13	2,6,10	13	C(Rev)	07/31/91
	QT	LXFL-529L	3.2-3.3	2.2-4.8	10(3)	0	0,13	2,6,10	13	A	07/22/91
LUNG (SCL)											
	TB	DMS 273	1.6-1.9	1.3-3.6	19(3)	0	0,9	2,6	9	A	07/31/91
	JR	DMS 114	2.8-7.5		19(3)	0	0,13	2,6,10	13	A	02/03/92
	JH	NCI-H69	4.2-6.8	2.8-8.2	19(3)	4*	4,15	4,8,12	15	A	08/05/87
	JK	NCI-H82	1.8-2.8	3-6.9	19(3)	0	0,9	2,6	9	A	04/17/87
MELANOMA											
	LO	LOX IMVI	1.6	1.2-2.5	10(3)	0	0,6	1,3	6	A	01/07/86
	QP	UACC-257	2.2-6.9	5.1	10(3)	0	0,13	2,6,10	13	A	07/22/91
					19(3)	0	0,17	2,6,10,14	17	B(Rev)	02/21/92
	QA	M14	5.4-16	3.7-6.8	19(3)	4*	4,15	4,8,12	15	A	07/29/91
	JQ	SK-MEL-5	5.7-13	3.3-5.6	19(3)	0	0,17	2,6,10,14	17	A	07/22/91
	QM	M19-MEL2	13-17	2.7-19	19(3)	0	0,13	2,6,10	13	A	07/22/91
	YP	SK-MEL-28	2-2.8	-	10(3)	0	0,13	2,6,10	13	A	07/31/91
	QN	UACC 62	2.5-5	9.8	19(3)	0	0,13	2,6,10	13	A	01/06/92
	QQ	SK-MEL-2	4.8-6.6	-	19(3)	0	0,13	2,6,10	13	A	01/10/92
	YF	MALME-3M	7.1-16.9	-	19(3)	4*	4,19	4,8,12,16	19	A	02/03/92
OVARIAN											
	JY	Ovcar-8	11-13	2.4-5.6	19(3)	0	0,13	2,6,10	13	A	07/22/91
	JX	Ovcar-5	2.2-3.9	-	19(3)	0	0,13	2,6,10	13	A	07/31/91
	YH	IGROV-1	5.3-15.8	-	19(3)	0	0,13	2,6,10	13	A	02/03/92
PROSTATE											
	JV	DU-145	6.3	4.2-6.3	19(3)	0	0,13	2,6,10	13	A	09/25/87
CENTRAL NERVOUS SYSTEM											
	UG	**U251**	3.9-6.7	2.2	10(3)	0	0,11	4,8	11	A	09/15/87
	QK	**SF-295**	1.7-1.8	-	10(3)	0	0,9	2,6	9	A	07/31/91
	TE	TE671	2.2-3.7	2.2-3.8	19(3)	0	0,9	2,6	9	A	07/14/87
LEUKEMIAS											
	BD	**MOLT-4**	1.8-7.5	-	10(3)	0	0,13	0,13	13	A	02/03/92

SPECIFIC TUMOR SUBRENAL CAPSULE PROTOCOL

	Tumor Code	Tumor Line	Doubling Times SC	Doubling Times SRC	Implant Size (OMU)	Initial Meas. Day	Weigh Days	Rx (Days)	Evaluation Day	Schedule Version	Schedule Date
COLON											
	YV	KM12a	2.5-2.8	2-5	10(3)	0	0,13	2,6,10	13	A	07/29/91
	JO	SW-620	1.8-3.1	1.6-8.1	10(3)	0	0,9	2,6	9	A	07/31/91
	TA	KM2OL2	3.5-4.3	2.7-7.1	10(3)	0	0,13	2,6,10	13	A	07/22/91
	C2	HT29	4.3-9.4	2.7	10(3)	0	0,19	4,8,12,16	19	B	04/30/87
					10(3)	0	0,13	2,6,10	13	C(Rev)	07/31/91
	QE	HCT-15	1.9-2.6	2.6	10(3)	0	0,13	2,6,10	13	A	07/31/91
	YK	HCT-116	1.7-3.3	1.8-4.3	19(3)	0	0,9	2,6	9	A	07/31/91
	YG	Colo-205	2.9-8.4	4.5-6.7	10(3)	0	0,13	2,6,10	13	A	01/07/92
	QI	HCC 2998	2.3-8.7	-	10(3)	0	0,13	2,6,10	13	A	02/03/92
RENAL											
	YE	CAKI-1	1.8-2.9	2.2-6.2	10(3)	0	0,13	2,6,10	13	A	07/18/91
	RG	RXF631	1.2-1.9	1.3-2.7	10(3)	0	0,9	2,6	9	A	07/22/91
	RF	RXF393	1.7-2.9	1.5-3.9	10(3)	0	0,13	2,6,10	13	A	07/22/91
	YJ	A498	3.3-4.1	2.9	19(3)	0	0,13	2,6,10	13	A	07/23/91
	RH	786-0	5.4-6.7	2.5-2.9	19(3)	0	0,13	2,6,10	13	A	07/22/91

Panel Tumors (3/1/90) in Bold
*Early Control Day